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Commentary

The Yin and Yang of vitamin D receptor (VDR) signaling in neoplastic progression: Operational networks and tissue-specific growth control

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ABSTRACT

Substantive evidence implicates vitamin D receptor (VDR) or its natural ligand 1 α ,25-(OH)₂ D₃ in modulation of tumor growth. However, both human and animal studies indicate tissue-specificity of effect. Epidemiological studies show both inverse and direct relationships between serum 25(OH)D levels and common solid cancers. VDR ablation affects carcinogen-induced tumorigenesis in a tissue-specific manner in model systems. Better understanding of the tissue-specificity of vitamin D-dependent molecular networks may provide insight into selective growth control by the seco-steroid, 1 α ,25-(OH)₂ D₃. This commentary considers complex factors that may influence the cell- or tissue-specificity of 1 α ,25-(OH)₂ D₃/VDR growth effects, including local synthesis, metabolism and transport of vitamin D and its metabolites, vitamin D receptor (VDR) expression and ligand-interactions, 1 α ,25-(OH)₂ D₃ genomic and non-genomic actions, Ca²⁺ flux, kinase activation, VDR interactions with activating and inhibitory vitamin D responsive elements (VDREs) within target gene promoters, VDR coregulator recruitment and differential effects on key downstream growth regulatory genes. We highlight some differences of VDR growth control relevant to colonic, esophageal, prostate, pancreatic and other cancers and assess the potential for development of selective prevention or treatment strategies.

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Keypoints

- Complex factors influence the cell- or tissue-specificity of vitamin D biological and growth effects, including local synthesis, metabolism and transport of vitamin D and its metabolites, vitamin D receptor (VDR) expression and ligand-interactions, 1 α ,25-(OH)₂ D₃ genomic and non-genomic actions,

1 α ,25-(OH)₂ D₃-mediated Ca²⁺ flux, kinase activation, VDR interactions with specific vitamin D responsive elements within target gene promoters, VDR coregulator recruitment and differential effects on key downstream target genes.

- Animal and in vitro studies show cell- or tissue-restricted vitamin D growth control.
- Epidemiological studies indicate vitamin D tissue-specific effects on neoplastic progression.
- E-cadherin and osteopontin (OPN) are functionally antagonistic VDR target genes that orchestrate the growth response to 1 α ,25-(OH)₂ D₃ in diverse tumor types.
- Consideration of 1 α ,25-(OH)₂ D₃-dependent signaling networks in a cell-lineage or tissue-specific context may shed light on its disparate growth regulatory effects and help exploit the promising therapeutic potential of VDR ligands, for selected cancers.

1. Introduction

1.1. The vitamin D endocrine system

Groundbreaking discoveries of the early 20th century elucidated vitamin D's essential role in calcium and phosphate homeostasis, bone mineralization and enabled major public health advances. The capacity of a novel fat-soluble vitamin that was distinct from vitamins A, B or C, for prevention of experimental

Abbreviations: 1 α ,25-(OH)₂ D₃, one alpha, 25 dihydroxyvitamin D₃; APC, adenomatous polyposis coli; CRC, colorectal cancer; DMBA, dimethylbenzanthracene; DR3-type, directly repeated arrangement of the hexameric binding sites with three spacing nucleotides; DRIP, Vitamin D receptor-interacting protein; ERK, extracellular signal-regulated kinase; GSK3 β , glycogen synthase kinase beta; HDAC, histone deacetylase co-repressor complex; MAPK, mitogen-activated protein kinase; NCoR, nuclear receptor co-repressor; NHL, non-Hodgkins lymphoma; OPN, osteopontin; RAC3, receptor activated coactivators 3; ROCK, Rho-associated coiled kinase; RXR, retinoid X receptor; SRC-1, steroid receptor coactivators-1; Tcf, T cell factor; TIF2, transcriptional intermediary factor 2; TPA, 12-O-tetradecanoylphorbol-13-acetate; VDRE, vitamin D response element; VDR, vitamin D receptor; WINAC, Williams syndrome transcription factor (WSTF) including nucleosome assembly complex.

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Rickets was shown in a seminal study by Mellanby [1]. Subsequently, Chick et al. demonstrated that clinical Rickets could be cured by dietary cod liver oil supplementation or sunlight exposure [2]. The Nobel prize in Chemistry was awarded to Dr Adolf Windaus in 1928, in recognition of his achievement in isolation of vitamin D and demonstration of its steroid structure [3]. In the 1930s, fortification of milk with vitamin D virtually eradicated Rickets from the United States, although it had previously been a highly prevalent crippling disease of childhood [4].

The mammalian form of vitamin D is a fat-soluble prohormone cholecalciferol (vitamin D₃) that may be generated endogenously by ultraviolet light-mediated metabolism of the precursor sterol 7-dehydrocholesterol, in the skin. Alternatively, vitamin D₃ may be obtained from dietary sources [5]. This prohormone (cholecalciferol) is hydroxylated to 25-hydroxycholecalciferol (25(OH)D₃) by hepatocyte 25-hydroxylase. Further hydroxylation by 1 α -hydroxylase (CYP27B1), into the main biologically active hormone, 1 α ,25-dihydroxycholecalciferol (1 α ,25-(OH)₂D₃ or calcitriol) occurs in the proximal renal tubule in a tightly regulated fashion [6]. 1 α ,25-(OH)₂D₃ then acts as a steroid chemical messenger in a diverse target tissues, in what is known as the vitamin D endocrine system [7]. To meet needs of bone mineralization, 1 α ,25-(OH)₂D₃ stimulates intestinal calcium and phosphate absorption, bone calcium and phosphate metabolism as well as renal calcium and phosphate reabsorption, by differential effects on osteoblasts, chondrocytes, renal and intestinal epithelia [8]. Furthermore, discovery of VDR expression in diverse normal human tissues including B and T lymphocytes, the hair follicle, muscle, adipose tissue, bone marrow and in cancer cells has widened the perceived scope of the vitamin D endocrine system, beyond bone homeostasis [7].

1.2. 1 α ,25-(OH)₂D₃ chemical structure and conformational relationships

1 α ,25-(OH)₂D₃, the active form of vitamin D, is a highly flexible molecule with a steroid carbon skeleton, involving 4 fused cyclopentanoperhydro-phenanthrene rings, A–D. Unlike other steroids, the 9–10 carbon bond is broken, thus creating a conformationally flexible molecule in which the “A” ring may rotate (Fig. 1). The molecule is technically classified as a seco-steroid. The spatial arrangements of principal functional components of the 1 α ,25-(OH)₂D₃ molecule comprise a hugely important determinant of its biological activities. *Cis-trans* isomerism influences stability and reactivity. The unusual degree of flexibility within 1 α ,25-(OH)₂D₃ enables synthesis of structural analogs (Fig. 1b) that elicit well-defined subsets of the vitamin D response (see below) [9,10].

1.3. Vitamin D transport

Normally, only 0.04% of 25(OH)D and 0.4% of 1 α ,25-(OH)₂D₃ are free in plasma, the remainder being tightly bound to either a vitamin D transporter protein (DBP) (85–88%; high affinity; dissociation constant [*K*_d] ~ 1 nM) [11] or albumin (12–15%; low affinity) [12]. Only free unbound vitamin D sterols are considered to be biologically active, since only the free form and not DBP-bound 1 α ,25-(OH)₂D₃ induces metabolic responses in target cells [13]. In addition to transport, DBP functions to maintain stable serum stores of vitamin D metabolites, modulate bioavailability and influence responsiveness of some end-organs [14]. 1 α ,25-(OH)₂D₃ binds to its “nuclear” receptor (VDR) with high affinity (dissociation constant value of [*K*_d] ~ 1 nM or lower) [15].

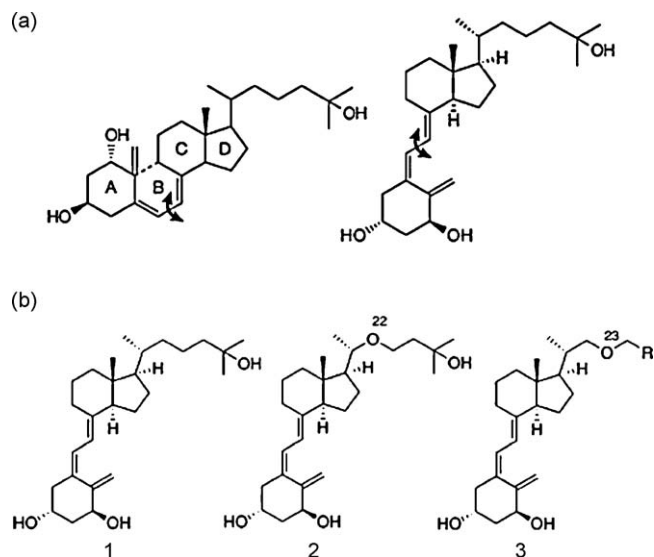


Fig. 1. Chemistry of 1 α ,25-(OH)₂D₃. 1 α ,25-(OH)₂D₃ is derived from the 4 cyclopentanoperhydro-phenanthrene ring structure (A, B, C, and D rings) for steroids. In 1 α ,25-(OH)₂D₃, the 9,10 carbon-carbon bond of ring B is broken between ring A and rings C and D (arrow, a) and the molecule is technically classified as a seco-steroid. The molecule may then rotate along the bond between ring A and rings C and D (arrow), to provide the structure of 1 α ,25-(OH)₂D₃ (a). Stepwise modification of the molecule, involving location of an oxygen atom at position 23 on the C and D ring side chain or removal of the terminal –OH group can have important biological effects (b).

1.4. The vitamin D receptor (VDR)

Free 1 α ,25-(OH)₂D₃ enters the cell and binds the vitamin D receptor (VDR) (Fig. 2a), that may be present in the cytoplasm, nucleus or partitioned between the cytoplasm and nucleus [16]. VDR is an endocrine member of the nuclear receptor superfamily [8] with high structural and ligand-binding homology across various species [6]. Ligands for VDR include bile acid metabolites as well as 1 α ,25-(OH)₂D₃ [17]. VDR has the same modular structure as other members of the nuclear receptor superfamily, including an N-terminal A/B region, a conserved DNA-binding domain, a flexible hinge region and a moderately conserved ligand-binding pocket that contains a dimerization interface and a ligand-dependent transcriptional activation domain, AF-2 [18] (Fig. 2a and b). Ligand binding induces a conformational change of the AF-2 region that allows dissociation of accessory proteins, exposure of the DNA-binding pocket and recruitment of coactivators [19]. Specific mutations that cause deletions, frameshift mutations, premature stop codons or splice site abnormalities that impede VDR expression or binding activity, effectively suppress key VDR actions [20].

1.5. 1 α ,25-(OH)₂D₃/VDR mediated genomic responses

The 1 α ,25-(OH)₂D₃/VDR complex functions to regulate gene transcription through heterodimerization with any of three retinoid X receptor (RXR) isoforms and binds to cognate vitamin D responsive elements (VDREs) in the promoter region of target genes. VDRE structures within promoter regions of primary 1 α ,25-(OH)₂D₃ regulated genes can vary [21]. However, the majority of known VDREs show a DR3-type structure comprising a directly repeated arrangement of hexameric binding sites with 3 spacing nucleotides [22]. This arrangement provides the most efficient interface for VDR/RXR heterodimer binding to core VDREs. Subclasses of DR3 VDREs show some sequence variation but their *in vivo* functionality is proportional to their *in vitro* binding affinity

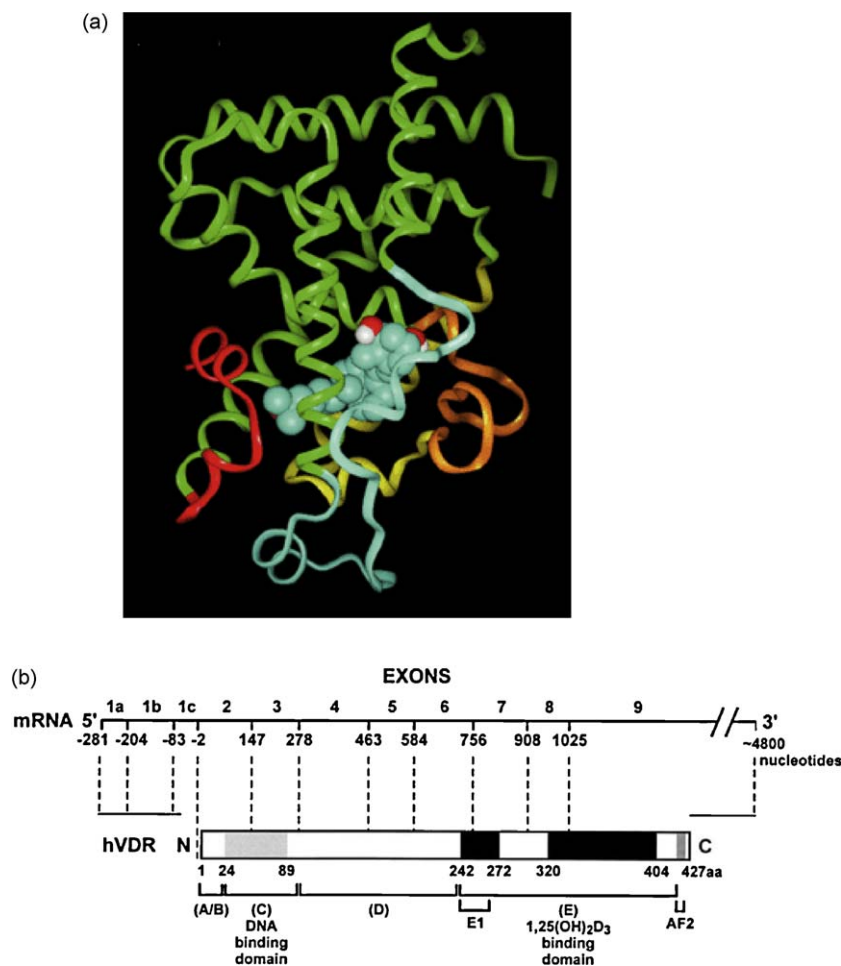


Fig. 2. Schematic representation of the vitamin D receptor (VDR) domain structure. (a) VDR protein backbone and $1\alpha,25-(\text{OH})_2 \text{D}_3$ ligand-binding pocket. The VDR protein backbone is represented by a ribbon. A space-filling representation of $1\alpha,25-(\text{OH})_2 \text{D}_3$ is shown within the VDR ligand-binding pocket by an atom-based structure. Conformational modification of the vitamin D side chain may influence ligand binding and transcriptional activity [125]. (b) The human VDR gene domain structure. The human VDR gene is composed of 9 exons that encode domains (A–F). Upon $1\alpha,25(\text{OH})_2 \text{D}_3$ binding to the hormone ligand-binding domain, VDR is stabilized by the phosphorylation of serine 51 in the DNA-binding domain and serine 208 in the hinge region. VDR associates with the retinoic acid receptor (RXR) through the dimerization domains in E/F. The $1\alpha,25-(\text{OH})_2 \text{D}_3$ -VDR-RXR complex binds to the vitamin D response elements (VDREs) through the DNA-binding domain in the promoters of target genes. Conformational change in VDR results in co-repressor dissociation and enables interaction of the AF2 transactivation domain with stimulatory coactivators, such as steroid receptor coactivators (SRCs), vitamin D receptor-interacting proteins complex and nuclear coactivators.

for VDR-RXR heterodimers [23]. Strongest affinity has been observed among class I DR3-type VDREs, including that present in the osteopontin (OPN) promoter [23]. $1\alpha,25-(\text{OH})_2 \text{D}_3$ may regulate genes that do not contain VDREs within their promoter regions, through non-genomic VDR actions (see below).

1.6. VDR/VDRE mediated coactivation or corepression of gene transcription

Regulation of gene expression by $1\alpha,25-(\text{OH})_2 \text{D}_3$ genomic signaling, is dependent upon the ability of VDR/RXR heterodimers to recruit coregulatory protein complexes [24] that may activate or repress target gene expression. Ligand triggered conformational change of VDR-RXR heterodimers results in dissociation of co-repressor proteins such as NCoR (nuclear receptor co-repressor) and facilitates the interaction with members of the CBP/p300 and p160 coactivator families including SRC-1 (steroid receptor coactivators-1), TIF2 (transcriptional intermediary factor 2), and RAC3 (receptor activated coactivators 3) [25]. DRIP (vitamin D receptor-interacting protein) cofactor complexes are also involved in parallel [19]. These coactivators bind ligand-activated VDR, induce a coactivator exchange in the transcriptional complex of VDR-responsive promoters [26] and enables opening of the

chromatin structure. These effects create an environment suitable for gene transcription [27].

VDR may also repress gene transcription. CYP27B1 catalyzes the metabolic activation of $1\alpha,25-(\text{OH})_2 \text{D}_3$ from its precursor [28] and is negatively regulated by $1\alpha,25-(\text{OH})_2 \text{D}_3$, in a cell-lineage-specific and tissue-restricted manner [29]. CYP27B1 and other genes including PTH [30] are thought to be suppressed by $1\alpha,25-(\text{OH})_2 \text{D}_3$ via negative vitamin D response elements (VDREs). Ligand-activated VDR binds to the 5' half site of negative VDREs [30]. VDR-RXR heterodimers interact with a VDR-interacting repressor (VDIR) at the E-box type element of negative VDREs, comprising a CANNTG-like motif. Such interactions at the E-box induce coregulator switching, involving dissociation of p300 coactivators but association of the histone deacetylase (HDAC) co-repressor complex, resulting in ligand-induced transrepression. Cell or tissue-specific transrepression of CYP27B1 by $1\alpha,25-(\text{OH})_2 \text{D}_3$ may involve multiple VDREs located in more distal promoter regions that enhance chromatin looping and interactions with protein super complexes of differing transcriptional abilities. Other mechanisms of transrepression involve the association between WINAC (Williams syndrome transcription factor (WSTF) including nucleosome assembly complex), a multifunctional, ATP-dependent chromatin-remodelling complex and chromatin [31] and

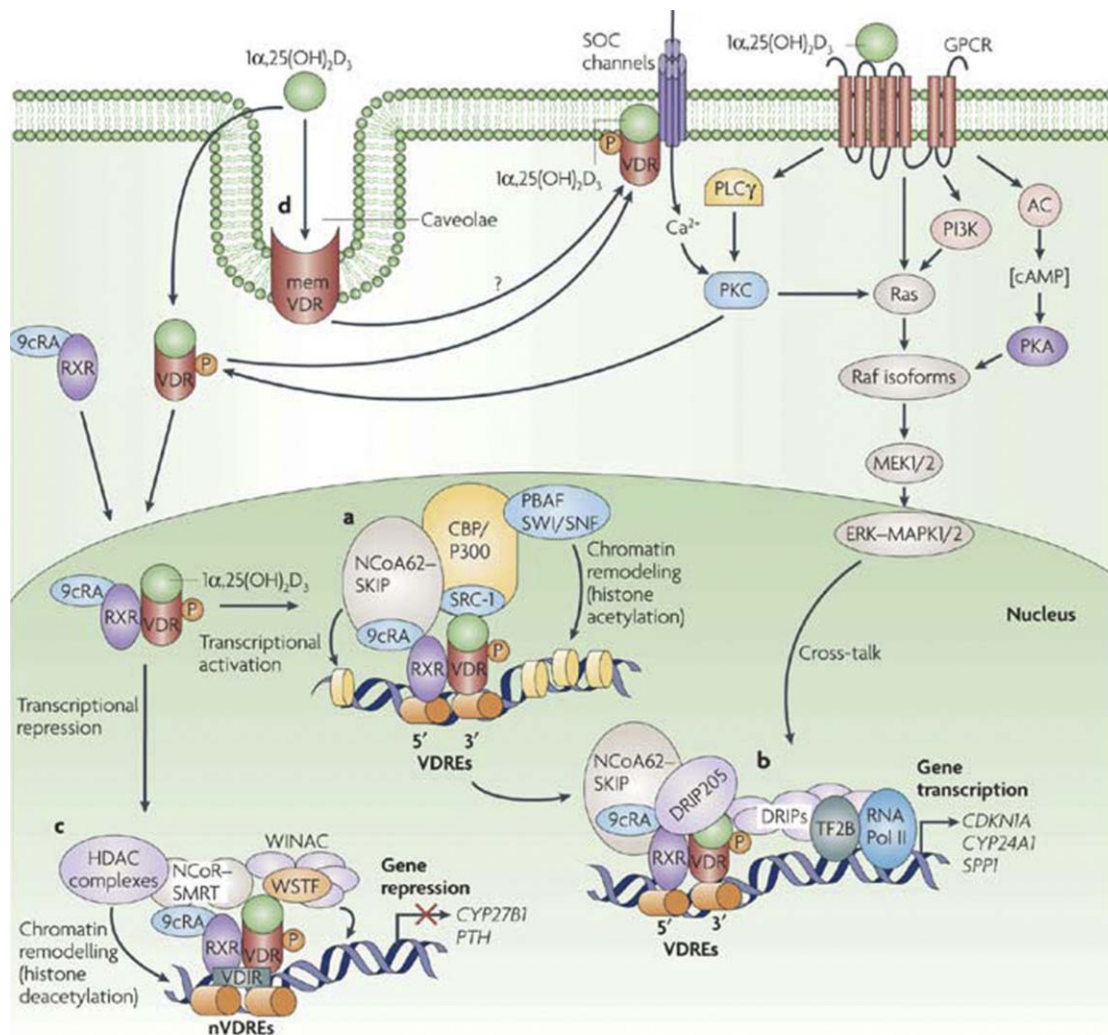


Fig. 3. Transcriptional regulation by vitamin D. Classical action of $1\alpha,25\text{-(OH)}_2\text{D}_3$ is mediated by binding of the VDR/RXR complex at VDREs. (a) Transcriptional activation involves the co-activator molecules SRCs, NCoA62, HATs, CBP p300 and other molecules to derepress chromatin. (b) Binding of DRIP to the AF2 domain attracts a complex containing transcription factor 2B (TF2B) and RNA polymerase II (RNA Pol II) for transcription initiation. The presence of the multiprotein complex facilitates increased gene transcription. (c) $1\alpha,25\text{-(OH)}_2\text{D}_3$ -mediated transcriptional repression involves VDR–RXR heterodimer association with VDR-interacting repressor (VDIR) bound to E-box-type negative VDREs (nVDREs), dissociation of the HAT co-activator and recruitment of histone deacetylase (HDAC) co-repressor. Williams syndrome transcription factor (WSTF) potentiates transrepression by interacting with a multifunctional, ATP-dependent chromatin-remodelling complex (WINAC) and chromatin. This leads to the repression of genes, such as *CYP27B1* (which encodes $1\alpha\text{-OHase}$) and *PTH* (which encodes parathyroid hormone). (d) Non-genomic, rapid actions of $1\alpha,25\text{-(OH)}_2\text{D}_3$ are through activation of mitogen-activated protein kinase (MAPK)–extracellular signal-regulated kinase (ERK) 1 and 2 cascade through the phosphorylation (P) and activation of Raf by protein kinase C (PKC), partly through induced changes of intra-cellular Ca^{2+} concentration (reproduced from Deeb et al. [126] with kind permission of Dr CS Johnston Roswell Park CI, New York and the publisher).

VDR-induced DNA methylation [32] (Fig. 3). These actions of the classical $1\alpha,25\text{-(OH)}_2\text{D}_3$ genomic response modulate synthesis and accumulation of new proteins and invoke appropriate cellular responses, over intervals of hours or days. These events may also be suppressed by protein synthesis inhibitors, such as actinomycin D or cycloheximide [16].

1.7. $1\alpha,25\text{-(OH)}_2\text{D}_3$ -mediated rapid or non-genomic responses

Notwithstanding the strong, non-covalent binding of $1\alpha,25\text{-(OH)}_2\text{D}_3$ to its cognate receptor, this active sterol can also elicit rapid responses in target cells of the vitamin D endocrine system. Effects include generation of calcium or ion flux [33], induction of second messenger systems [34] and activation of cytosolic kinases [35]. $1\alpha,25\text{-(OH)}_2\text{D}_3$ may promote rapid Ca^{2+} influx from the extracellular space through voltage-independent channels in rat osteosarcoma cells [36], release Ca^{2+} from intra-cellular stores in osteoblasts [37] and activate protein kinase pathways that may be Ca^{2+} -dependent or -independent [37,38]. These effects occur

rapidly within minutes and are considered incompatible with mechanisms involving alterations in gene transcription and protein synthesis [33]. Involvement of VDR in this pathway remains controversial, since rapid actions of $1\alpha,25\text{-(OH)}_2\text{D}_3$ may be invoked in cells that lack VDR [39]. However, in various cell types rapid responses can be mediated by $1\alpha,25\text{-(OH)}_2\text{D}_3$ acting through a population of classical VDR molecules acting outside the cell nucleus, associated with caveolae of the plasma membrane [40].

Importantly, analogs of different vitamin D isomers that are locked in *cis*- or *trans*-conformations have been synthesized which can preferentially elicit rapid non-genomic and/or genomic responses [9,10].

1.8. $1\alpha,25\text{-(OH)}_2\text{D}_3$ dual regulation of gene expression by genomic and rapid non-genomic pathways

There is compelling evidence that the rapid non-genomic activation of signal transduction pathways by $1\alpha,25\text{-(OH)}_2\text{D}_3$ can interact with and modulate VDR-dependent gene transcription

[41,42]. While the $1\alpha,25-(\text{OH})_2 \text{D}_3$ -liganded RXR–VDR hetero-complex selectively recognizes VDREs in the promoter regions of osteopontin (OPN) [43] and osteocalcin (OCN) [44] genes, the steady state levels of OPN and OCN mRNA can also be modulated by $1\alpha,25-(\text{OH})_2 \text{D}_3$ rapid non-genomic actions [45]. Furthermore, antagonism of the non-genomic pathway blocks $1\alpha,25-(\text{OH})_2 \text{D}_3$ -mediated OCN expression [41]. $1\alpha,25-(\text{OH})_2 \text{D}_3$ rapid activation of cytosolic kinases may phosphorylate critical coactivators resulting in modulation of VDR-dependent gene transcription [46]. By non-genomic actions, $1\alpha,25-(\text{OH})_2 \text{D}_3$ can modulate a repertoire of cytosolic kinases and second messenger systems that show some level of cell- or tissue-specificity [38], e.g. activation of phospholipase A2 in chondrocytes [47] and protein kinase A in enterocytes [48]. By cross-talk with VDR/VDRE regulation of gene transcription, these membrane-mediated kinase cascades may influence cell-specific biological responses to $1\alpha,25-(\text{OH})_2 \text{D}_3$, involved diverse physiological and pathobiological processes [7].

1.9. Tissue- and cell-specificity of vitamin D biological effects

Most tissues express the receptor for $1\alpha,25-(\text{OH})_2 \text{D}_3$ (VDR) and renal tubules, skin, bone, brain, breast, colon and prostate also contain the enzyme CYP27B1, required for converting the major circulating metabolite of vitamin D, $25(\text{OH})\text{D}$ to $1\alpha,25-(\text{OH})_2 \text{D}_3$ [49]. Notwithstanding the wide distribution of VDR and CYP27B1, $1\alpha,25-(\text{OH})_2 \text{D}_3$ shows highly tissue-specific functional effects on hormone secretion, immune function, cell differentiation and growth. For example, $1\alpha,25-(\text{OH})_2 \text{D}_3$ inhibits PTH secretion in the parathyroid glands [30] but stimulates pancreatic β -cell insulin secretion [50], inhibits adaptive immunity [51] but enhances some innate immune responses [52], inhibits differentiation of B lymphocytes [53] but enhances keratinocyte differentiation [54].

$1\alpha,25-(\text{OH})_2 \text{D}_3$ -mediated growth effects may show similar cell-specificity. For example, $1\alpha,25-(\text{OH})_2 \text{D}_3$ has antiproliferative effects in some neoplastic cells [55] but induces a spectrum of growth responses in others. The PC-3 and DU-145 prostate cancer cell lines for example are not significantly inhibited by physiologically relevant doses of $1\alpha,25-(\text{OH})_2 \text{D}_3$ [56]. Furthermore, at low or physiological concentrations, $1\alpha,25-(\text{OH})_2 \text{D}_3$ may promote proliferation of monocytes [57] or keratinocytes [58]. Effects upon anchorage-independent growth and invasion may show similar cell-specificity. For example, $1\alpha,25-(\text{OH})_2 \text{D}_3$ or analogs inhibited anchorage-independent growth of prostate cancer cells [59] and suppressed invasion through Matrigel by these cells [59] and neuroblastoma cells [60]. Conversely, vitamin D or analogs may also enhance anchorage-independent growth [61] and promote 12-O-tetradecanoylphorbol-13-acetate (TPA) induced neoplastic transformation in JB6 epidermal cells [62].

These observations do not support uniform growth or other biological responses to vitamin D exposure. Rather, cell- or tissue-specific processes, could modulate the initiating $1\alpha,25-(\text{OH})_2 \text{D}_3$ signal to provide different functional outcomes.

2. The Yin and Yang of $1\alpha,25-(\text{OH})_2 \text{D}_3$ growth regulatory signaling

Expression profiling has identified diverse targets of $1\alpha,25-(\text{OH})_2 \text{D}_3$ non-genomic or genomic actions including G-coupled receptors, inter- and intra-cellular signaling genes, cell-cycle regulators, metabolic function moieties, extracellular matrix components and cell adhesion molecules [63]. From within these networks, $1\alpha,25-(\text{OH})_2 \text{D}_3$ not only regulates bone mineralization but also modulates growth and differentiation [64]. Such diversity of biological effect could be achieved in part through genomic/non-genomic cross-talk, molecular networks or transcriptomes impli-

cated in lineage specialization or effects on target genes with context-specific functions.

Within such modular networks, osteopontin (OPN) and E-cadherin play important roles in growth responses to vitamin D [65,66]. Osteopontin (OPN) is a key vitamin D target gene, regulated by $1\alpha,25-(\text{OH})_2 \text{D}_3$ -mediated genomic [26] and non-genomic mechanisms [45,67]. OPN is an extracellular matrix glycoprophosphoprotein implicated in osteoblast differentiation [45] but is also a central effector of vitamin D – mediated anchorage-independent growth [68]. OPN may abrogate the adhesion requirement for cell growth and enhance cell invasion through Matrigel by activation of Ran GTPase (RAN) [69]. $1\alpha,25-(\text{OH})_2 \text{D}_3$ transcriptional regulation of OPN involves VDR/RXR heterodimer binding and recruitment of coregulators including SRC-1, -2, -3, CBP, p300 and DRIP205 to VDREs within the OPN promoter [26]. Mutation at one or both VDRE sites in the rat OPN promoter substantially suppresses $1\alpha,25-(\text{OH})_2 \text{D}_3$ -mediated transcription of a OPN-promoter luciferase reporter construct [70]. However, OPN mRNA may also be activated by VDR non-genomic actions, involving Ca^{2+} -influx and rapid activation of the small GTPase, RhoA and its effector, Rho-associated coiled kinase (ROCK) [67]. Effects of this non-genomic pathway on OPN protein expression are unclear [67]. RhoA–ROCK activation of OPN mRNA in smooth muscle cells is Erk dependent and may be suppressed by the MEK1 inhibitor, PD98059 [71].

E-cadherin is induced by $1\alpha,25-(\text{OH})_2 \text{D}_3$ non-genomic rapid actions [67] and suppresses cell growth, partly by inhibition of β -catenin transcriptional activity [72,73]. Free β -catenin that is sequestered by E-cadherin, is rapidly phosphorylated by glycogen synthase kinase3 β (GSK3 β) in the adenomatous polyposis coli (APC)/axin/GSK-3 β /casein kinase I complex and is subsequently ubiquitinated and degraded. Loss of this function enables β -catenin accumulation and translocation to the nucleus where it modulates the expression of Tcf/Lef-1-target genes implicated in cell proliferation [74]. $1\alpha,25-(\text{OH})_2 \text{D}_3$ induction of E-cadherin involves transcription-independent promotion of Ca^{2+} -influx and consequent activation of RhoA–ROCK signaling. Subsequent to these events, induction of p38/MAPK–MSK1 signaling upregulates E-cadherin and inhibits β -catenin/Tcf transcriptional activity [67]. Hence, OPN and E-cadherin are functionally antagonistic growth regulatory genes that are modulated by $1\alpha,25-(\text{OH})_2 \text{D}_3$ through overlapping but distinct molecular mechanisms.

2.1. Growth signaling through E-cadherin and OPN: functional outcomes of $1\alpha,25-(\text{OH})_2 \text{D}_3$ treatment

E-cadherin and OPN are reciprocally regulated through β -catenin/Tcf and related signaling pathways [75] to provide high and low levels respectively, in quiescent normal tissue [76,77]. Disturbance of this equilibrium in early stages of multistep tumorigenesis may have phenotypic effects on cell adhesion [78], migration [76] and invasion [68]. Aberrant expression of these genes in early tumorigenesis may influence the subsequent development of abnormal molecular circuitry in evolving cancer cells.

To investigate involvement of the E-cadherin/OPN equilibrium in $1\alpha,25-(\text{OH})_2 \text{D}_3$ growth control, Xu et al. used parental cell lines and stably transformed subclones with variable constitutive expression of these genes [70]. Parental R37 mammary cells highly express E-cadherin, have an epithelial-like morphology, have low levels of Tcf-1 indicative of low level β -catenin signaling activity and weak expression of OPN. Subclones were raised by stable transfection of R37 cells with metastatic tumor DNA fragments (R37 Met-DNA; [C9] cells) that upregulates OPN [79]. Subclones were also raised by transfection of parental R37 cells with OPN cDNA in sense or antisense orientations respectively, in

expression vectors. R37 Met-DNA [C9] cells express high levels of OPN and Tcf-1, low levels of E-cadherin, have a spindle-like morphology and are invasive [75]. In these cells, OPN is considered to be the direct effector of Met-DNA, in promotion of invasion or metastasis [80]. In this model system, $1\alpha,25-(\text{OH})_2 \text{D}_3$ and novel A-ring modified vitamin D analogs influenced the balance of these antagonistic VDR-dependent signals. All treatments upregulated E-cadherin, suppressed β -catenin transcriptional activity and β -catenin nuclear localization, consistent with growth-inhibition. However, all treatments also upregulated OPN that may be implicated in neoplastic transformation and invasion.

Although molecular cross-talk was observed, growth effects induced by $1\alpha,25-(\text{OH})_2 \text{D}_3$ or analogs appeared dependent upon the constitutive balance of these functionally antagonistic molecules, in target cells. VDR ligands significantly increased migration or invasion only in those cells with high constitutive OPN and low E-cadherin [70]. This finding suggests that the pre-treatment activity state of antagonistic VDR-dependent molecules may influence cell-specific $1\alpha,25-(\text{OH})_2 \text{D}_3$ growth responses. These fundamental studies may provide greater understanding of $1\alpha,25-(\text{OH})_2 \text{D}_3$ growth effects relating to cancer, in whole animal studies as well as clinical or epidemiological surveys.

2.2. Tumorigenesis in VDR knockout mice

Development of a null mouse model has provided an important tool for study of $1\alpha,25-(\text{OH})_2 \text{D}_3$ /VDR functional roles in tumor biology. VDR null mice appear developmentally normal at birth but manifest growth abnormalities, alopecia and infertility from the time of weaning [81]. Mice deficient in VDR or key components of the vitamin D synthetic pathway do not manifest any increase of sporadic tumorigenesis [82]. However, VDR ablation alters susceptibility to chemically induced carcinogenesis in a tissue-specific manner [83]. Administration of DMBA together with medroxyprogesterone induced increased formation of skin tumors and mammary hyperplasia in VDR knockout vs. control mice [83]. Although differences were observed in development of thymic lymphomas, lymphoblastic leukemias and mammary tumor histopathology, VDR status did not affect overall non-epidermal tumor incidence. No effects were observed on tumorigenesis in the ovary, uterus, liver or lung, despite expression of the VDR in these tissues. These findings provide strong evidence that VDR signaling alters chemically induced carcinogenesis in a manner that is tissue-specific but unrelated to VDR expression, *in vivo* [83].

2.3. Vitamin D exposures and human cancer risk

Associations between sunlight exposure, dietary histories and tumor incidence in various epidemiological surveys imply an important role for vitamin D in lifetime cancer risk and/or survival [84]. For example, mortality from colon cancer was found to be higher in geographical regions of the United States with low sunlight exposure [85] while decreased colon [86], breast [87] and prostate cancer risk [88] were reported in high sunlight areas. Inverse relationships between dietary vitamin D intake and breast cancer have been reported [89,90] although findings for colorectal cancer appeared inconsistent [91,92] and no clear associations were found for prostate cancer [93,94]. The relationship between sunlight exposure and risk of lymphoma has also been controversial. Studies have shown positive [95,96], inconsistent [97] or inverse [98] relationships between estimated solar ultraviolet exposure and non-Hodgkins lymphoma (NHL). No clear association between dietary intake of vitamin D and risk of NHL, diffuse large B-cell lymphoma, chronic lymphocytic leukemia or follicular lymphoma were found [99]. Study participants' histories of sun

exposure or intake of foods that are high in vitamin D may however be subject to systematic recall error and associated bias.

The vitamin D metabolite 25-OHD has a long half-life and serum levels may provide a robust biomarker of vitamin D status [100]. Subjects with high levels of 25-OHD were found to have a lower incidence of colorectal cancer (CRC), in both women and men [101,102]. Freedman and colleagues confirmed an inverse association between serum 25-OHD levels and CRC risk and demonstrated that the highest percentile serum 25-OHD levels had a CRC relative risk (RR) of 0.28 [103]. No clear association with total cancer mortality was observed, however [103]. Associations between 25-OHD levels, breast [104,105] and total prostate cancer [106–108] have been inconsistent. In a nested case–control study involving 270 incident lymphoid cancer cases and 538 controls from a cohort of 29,133 Finnish male smokers, serum 25-OHD levels were not associated with the risk of overall lymphoid cancers, NHL or multiple myeloma [109]. However, high serum 25-OHD levels were associated with increased risk of pancreatic cancer in 200 Finnish cases and 400 controls, from this same total cohort [110]. In a further nested case–control study, high prediagnostic serum 25-OHD levels had increased risk of aggressive prostate cancer in 749 cases and 781 control subjects from the US Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial [108].

In the nested case–control study design, non-diseased subjects from whom the controls are selected may not be fully representative of the healthy population [111]. In a prospective cohort study of >700 apparently healthy adults from the Linxian region of China, higher serum 25(OH)D concentrations were associated with significantly increased risk of development of esophageal squamous dysplasia [112] and invasive squamous carcinoma [113].

These epidemiological studies show positive and negative associations between vitamin D status and common solid cancers in various populations and allude to tissue-specificity of effect. At present, any chemopreventive benefits of higher serum 25(OH)D levels for colorectal cancer [102,103] may require to be weighed against potential increased risks of esophageal squamous dysplasia [112] or cancer [113], pancreatic cancer [110] or aggressive prostate cancer [108]. Within interacting molecular networks that influence tissue-specific responses to $1\alpha,25-(\text{OH})_2 \text{D}_3$, a dynamic equilibrium of positive or negative growth signals may determine ultimate outcomes, although key molecules can have specific effects upon development of neoplastic phenotype.

2.4. Tissue-specific VDR operational networks and cancer risk

The VDR growth regulatory equilibrium involving E-cadherin and OPN is disturbed during stepwise evolution of many human cancers [77,114] although there are important tissue-specific differences. In the colon for example, adenomatous polyps represent the commonest identifiable premalignant lesion. These colonic adenomas have E-cadherin/OPN expression patterns resembling that of normal mucosa, namely preservation of E-cadherin [115] and low or undetectable OPN [116]. Hence, in these lesions, high level serum [25(OH)D] could further increase E-cadherin tumor suppressor activity, set against low level OPN with an overall effect of growth restraint. By this rationale, persistently high serum [25(OH)D] could impede neoplastic progression of colonic adenomas which are common in asymptomatic Western populations [117], with ultimate reduction of CRC incidence. Conversely, preneoplastic squamous mucosal hyperplasia or dysplasia are common in high risk areas for esophageal cancer [118]. Unlike colonic adenomas, these lesions are characterised by early suppression of E-cadherin [119] and upregulation of OPN [120]. In such populations, higher serum [25(OH)D] levels are associated with increased esophageal

squamous cancer risk [113]. Potentially, high $1\alpha,25-(\text{OH})_2 \text{D}_3$ exposure could enhance the predominance of OPN growth-promoting signals and associated Ran activity [69] in these lesions, beyond the threshold level required for invasion and neoplastic progression. Neoplasms of the pancreas and prostate are characterised by similar disequilibrium involving low E-cadherin and high OPN [121–124] which could potentially be related to the direct associations between serum $25(\text{OH})\text{D}$ levels and risks of pancreatic cancer or aggressive prostate cancer [67,108,110].

Tissue-specific $1\alpha,25-(\text{OH})_2 \text{D}_3$ growth responses may involve complex non-genomic/genomic cross-talk and modulation of downstream signals by distinct lineage-specific expression patterns of the human transcriptome. However, the E-cadherin/OPN expression balance may provide a useful biologically based marker of this complexity, implicated in $1\alpha,25-(\text{OH})_2 \text{D}_3$ growth responses.

2.5. Towards conditional targeting strategies

The above studies address fundamental mechanisms and link tissue-specific differences of VDR growth regulatory networks, particularly involving OPN and E-cadherin to epidemiological associations between serum $25(\text{OH})\text{D}$ levels and cancer.

Although genomic effects of $1\alpha,25-(\text{OH})_2 \text{D}_3$ are partly implicated in induction of OPN [7], they appear dispensable for induction of E-cadherin in colorectal cells [67]. $1\alpha,25-(\text{OH})_2 \text{D}_3$ induces transcription-independent Ca^{2+} influx and activation of RhoA-ROCK, in the presence VDR. Thus activated, RhoA-ROCK upregulates p38 MAPK and MSK1, leading to substantive induction of E-cadherin mRNA and protein, inhibition of β -catenin/Tcf transcriptional activity and suppression of cell proliferation [67]. OPN mRNA may also be regulated by $1\alpha,25-(\text{OH})_2 \text{D}_3$ through transcription-independent, Ca^{2+} -dependent RhoA/ROCK activation although the response appears less robust than that of E-cadherin. $1\alpha,25-(\text{OH})_2 \text{D}_3$ induced 30-fold induction of E-cadherin mRNA but less than 10-fold upregulation of OPN mRNA within 8 h [67]. Downstream of Rho/ROCK, p38/MSK1 is required for induction of E-cadherin [67]. Conversely, in smooth muscle cells RhoA/ROCK activation of ERK is implicated in upregulation of OPN mRNA.

Additional work is required to explore mechanistic issues involving $1\alpha,25-(\text{OH})_2 \text{D}_3$ rapid vs. genomic effects, regulation of signaling kinases and differential activation of E-cadherin/OPN growth regulatory genes. Rational design of combination therapies that allows activation of E-cadherin without upregulation of OPN may be a useful target for tissue-specific pharmacotherapeutics, particularly for preneoplastic states characterised by high constitutive OPN. Such future advances may provide a rationale for improved prevention and treatment of different cancers, through VDR mediated growth control.

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